

# PATENT COOPERATION TREATY

**PCT**

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
United States Patent and Trademark  
Office  
Box PCT  
Washington, D.C. 20231  
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

<b>Date of mailing (day/month/year)</b> 11 November 1999 (11.11.99)	
<b>International application No.</b> PCT/IB99/00520	<b>Applicant's or agent's file reference</b> 7070/VB
<b>International filing date (day/month/year)</b> 25 March 1999 (25.03.99)	<b>Priority date (day/month/year)</b> 26 March 1998 (26.03.98)
<b>Applicant</b> RUBINGH, Donn, Nelson et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:  
27 September 1999 (27.09.99)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

<b>The International Bureau of WIPO</b> 34, chemin des Colombettes 1211 Geneva 20, Switzerland	<b>Authorized officer</b> C. Carrié
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/57, 9/54, A61K 7/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/49057</b> <b>(43) International Publication Date:</b> 30 September 1999 (30.09.99)
<b>(21) International Application Number:</b> PCT/IB99/00520 <b>(22) International Filing Date:</b> 25 March 1999 (25.03.99) <b>(30) Priority Data:</b> 60/079,477 26 March 1998 (26.03.98) US <b>(71) Applicant (for all designated States except US):</b> THE PROCTER & GAMBLE COMPANY [US/US]; One Procter & Gamble Plaza, Cincinnati, OH 45202 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> <u>RUBINGH</u> , Donn, Nelson [US/US]; 8224 Sheed Road, Cincinnati, OH 45247 (US). <u>SIKORSKI</u> , Elizabeth, Ellen [US/US]; 5687 Genevieve Place, Fairfield, OH 45014 (US). <u>CORREA</u> , Paul, Elliott [US/US]; 4245 Miami Trails Lane, Cincinnati, OH 45252 (US). <b>(74) Agents:</b> REED, T., David et al.; The Procter & Gamble Company, 5299 Spring Grove Avenue, Cincinnati, OH 45217-1087 (US).		<b>(81) Designated States:</b> AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> SERINE PROTEASE VARIANTS HAVING AMINO ACID DELETIONS AND SUBSTITUTIONS  <b>(57) Abstract</b>  The present invention relates to variants of serine proteases having decreased immunogenicity relative to their corresponding wild-type proteases. More particularly, the present invention relates to variants having a modified amino acid sequence of a wild-type amino acid sequence, wherein the modified amino acid sequence comprises a deletion and, optionally, a substitution of one or more specifically identified positions corresponding to subtilisin BPN'. The invention further relates to mutant genes encoding such variants and cleaning and personal care compositions comprising such variants.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>7070/VB</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/IB 99/ 00520</b>	International filing date (day/month/year) <b>25/03/1999</b>	(Earliest) Priority Date (day/month/year) <b>26/03/1998</b>
Applicant <b>THE PROCTER &amp; GAMBLE COMPANY et al.</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

### 1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

## INTERNATIONAL SEARCH REPORT

International Application No

/IB 99/00520

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/57 C12N9/54 A61K7/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 10755 A (NOVONORDISK AS) 25 June 1992 (1992-06-25)	1-10
Y	the whole document especially claim 1-14 ---	9, 10
A	WALSH B AND HOWDEN M: "A method for detection of IgE binding sequences of allergens based on a modification of epitope mapping" JOURNAL OF IMMUNOLOGICAL METHODS., vol. 121, 1989, pages 275-289, XP002112551 ELSEVIER SCIENCE PUBLISHERS B.V., AMSTERDAM., NL ISSN: 0022-1759 cited in the application the whole document --- -/--	1-10

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

19 August 1999

Date of mailing of the international search report

01/09/1999

Name and mailing address of the ISA

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## INTERNATIONAL SEARCH REPORT

International Application No

/IB 99/00520

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 88 08028 A (GENEX CORP) 20 October 1988 (1988-10-20)	1-4, 6-8
Y	claims 1-20 ----	9, 10
A	WO 96 09396 A (UNIV MARYLAND) 28 March 1996 (1996-03-28) -----	

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 99/00520

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9210755 A	25-06-1992	AT 170630 T	15-09-1998
		AU 9052891 A	08-07-1992
		CA 2095852 A	06-06-1992
		DE 69130113 D	08-10-1998
		DE 69130113 T	12-05-1999
		EP 0561907 A	29-09-1993
		FI 932561 A	04-06-1993
		JP 6502994 T	07-04-1994
		US 5766898 A	16-06-1998
WO 8808028 A	20-10-1988	DE 353250 T	28-05-1998
		DK 496489 A	06-10-1989
		EP 0353250 A	07-02-1990
		EP 0916732 A	19-05-1999
		JP 2502874 T	13-09-1990
		US 4990452 A	05-02-1991
		US 5260207 A	09-11-1993
WO 9609396 A	28-03-1996	US 5567601 A	22-10-1996
		AU 693552 B	02-07-1998
		AU 2433595 A	09-04-1996
		BR 9508981 A	30-12-1997
		CA 2200563 A	28-03-1996
		CN 1166183 A	26-11-1997
		EP 0804592 A	05-11-1997
		JP 10506277 T	23-06-1998
		NZ 285286 A	26-06-1998

REC'D 05 JUL 2000

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 7070/VB	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/IB99/00520	International filing date (day/month/year) 25/03/1999	Priority date (day/month/year) 26/03/1998
International Patent Classification (IPC) or national classification and IPC C12N15/57		
Applicant THE PROCTER & GAMBLE COMPANY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand  27/09/1999	Date of completion of this report  30.06.2000
Name and mailing address of the international preliminary examining authority:   European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer  Kalsner, I  Telephone No. +49 89 2399 8708 



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/IB99/00520

**I. Basis of the report**

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

**Description, pages:**

1-86 as originally filed

**Claims, No.:**

1-10 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

**IV. Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.  
☐ paid additional fees.  
☐ paid additional fees under protest.  
☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/IB99/00520

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☐ complied with.
- ☒ not complied with for the following reasons:

**see separate sheet**

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☒ all parts.
- ☐ the parts relating to claims Nos. .

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes:	Claims 1-10
	No:	Claims
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-10
Industrial applicability (IA)	Yes:	Claims 1-10
	No:	Claims

2. Citations and explanations

**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

**Ad Section IV: Lack of unity of invention**

An international application must relate to one invention only or to a group of inventions so linked as to form a single general inventive concept.

Unity of invention is fulfilled only when there is a technical relationship among the inventions involving one or more of the same special technical features, special technical features being such features that define a contribution over which each of the claimed inventions, considered as a whole, makes over the prior art.

The following documents are referred to:

D1...WO-A-96 09396

D2...WO-A-92 10755

The present application relates to serine proteases which have been modified by deleting one or more amino acids at positions 70-84 of subtilisin BPN'. These variants are said to be less immunogenic than their wild-type counterparts.

Claim 1 refers to a serine protease mutant being characterised by a modified amino acid sequence of a wild-type amino acid sequence, wherein the modified amino acid sequence comprises a deletion of one or more amino acid at one or more of positions 70-84. Claim 5 defines 10 specific variants.

Variants of serine proteases are well known in the state of the art. For instance, D1 discloses serine protease variants having deletions at positions 75-83 which are calcium free and retain their enzymatic activity and stability. D2 discloses serine proteases which have been modified for reduced immunogenicity.

The feature common to the different variants claimed in claims 1-5 is the fact, that they comprise deletions at positions 70-84. This feature is neither new nor inventive in view of the prior art. This concept/relationship, therefore cannot be accepted to constitute a special technical feature as defined above as it does not define a contribution which each of the different claimed inventions, considered as a whole, makes over the prior art.

Hence, the various serine protease mutants are not considered unitarian.

According to Rule 68.1 PCT, however, the IPEA chooses not to invite the applicant to restrict or pay additional examination fees.

**Ad Section V: Reasoned statement with regard to novelty, inventive step or industrial applicability**

**1) Documents**

D1...WO-A-96 09396

**2) Novelty and inventive step**

- 2.1) **Claim 1** relates to a variant of a serin protease which is characterised by a modified amino acid sequence of a wild-type amino acid sequence, wherein said modified amino acid sequence comprises a deletion of one or more of positions 70-84 corresponding to subtilisin BPN', whereas the variant is not  $\Delta$  75-83 or  $\Delta$  71.

As none of the cited prior art discloses variants of serin proteases as defined in claim 1 **claim 1** and dependent claims (**claims 2-7 and 8-10**) are considered to meet the requirements of Art.33(2)PCT.

- 2.2) **Claim 1**, however, does not meet the requirements of Art. 33(3) PCT for the following reasons:

D1 which is considered to represent the closest prior art, discloses subtilisin variants comprising deletions of amino acids in the region of amino acids 70-89, one specific mutant lacking amino acids 75-83. These mutants are reported to retain enzyme activity.

The provision of further subtilisin variants which have mutations in the very same region is not considered to involve an inventive step. Hence **claim 1** and dependent claims thereof (**claims 2-7**) as well as **claim 8** which relates to genes

encoding such variants are not considered to meet the requirements of Art. 33(3) PCT.

- 2.3) **Claims 9 and 10** do not meet the requirements of Art. 33(3) PCT as the provision of personal care compositions comprising variants of serine proteases which are themselves not considered inventive seems to be obvious for the skilled person.

**Ad Section VIII: Certain observations on the international application**

- 1) **Claim 1** does not meet the requirements of 6 PCT for the following reasons:

According to the description (p. 5, 2<sup>nd</sup> par.) a characterising feature of the claimed subtilisin variants is their decreased immunogenicity when compared to their wild-type counterparts. As this feature is not reflected in claim 1, the claim is not considered clear in the sense of Art. 6 PCT.

- 2) In addition, the application does not meet the requirements of Art. 5 PCT as the description is devoid of any example comparing the immunogenicity of a mutant serine protease with a wild-type serine protease.

It should be noted, that, in order to qualify as a characterising feature, the "decreased immunogenicity" of the claimed subtilisin variants has to be proven by experiments comparing their immunogenicity with the immunogenicity of the corresponding wild-type subtilisin.